The role of actinomycetes in the terrestrial degradation of wood

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Abstract

Monocultures of *Streptomyces* spp. were used to evaluate the wood degradation potential of actinomycetes in *Alnus rubra* Bong. and *Pseudotsuga menziesii* (Mirb.) Franco. The rate and extent of degradation was assessed by weight loss and by micromorphological studies using scanning electron microscopy. Only slight weight losses were produced in the wood. The micromorphology of wood colonized by *Streptomyces* showed that all strains that were examined rapidly colonized wood blocks, however, conventional staining and light microscope techniques did not reveal the extent of vegetative mycelium actually present. Scanning electron microscopy demonstrated the presence of extensive vegetative mycelium and intact reproductive structures within the wood, but there was little microscopic evidence of degradation of wood cell walls by streptomycetes, even after 24 weeks of incubation. The interactions between *Streptomyces* and fungi were studied in pure and mixed cultures and it was shown that streptomycetes could inhibit the attack of decay fungi during the biodeterioration of wood. In agar plate decay tests, more wood decay, as measured by weight loss of test blocks, usually resulted from the action of the decay fungus alone than from combined attack of *Streptomyces* and a decay fungus.

Actinomycetes comprise a group of unicellular filamentous microorganisms (about 1μ in diameter, with monopodial, more rarely dichotomous, branching) that produces colonies of radiating structure (18). They have frequently been looked upon as a separate group of microorganisms that occupies a position between the true fungi and the true bacteria. However, recent evidence and rapidly accumulating information concerning the chemical composition of actinomycetes tend to emphasize the close relation of actinomycetes to the true bacteria (19). It has even been suggested that they form the original prototypes from which both fungi and bacteria have been derived (17).

Actinomycetes are found in all natural terrestrial and aquatic environments. There are many reports of their occurrence as members of the wood degradation microflora (2,4,12), but their importance as independent agents of cell wall degradation has not been extensively investigated. The recent emphasis on the role of bacteria in wood degradation (15), the consistent isolation of actinomycetes from wood stored in a variety of natural environments in ecological studies (3, 8), and the association of actinomycetes with preservative-treated wood (10) have encouraged a greater interest in the role of this group of microorganisms in wood degradation.

The role of actinomycetes in biodeterioration has been reviewed by Williams (20), and the literature on actinomycete colonization of wood in soil and marine environments has been reviewed by King, Eaton, and Baecker (12). These reviews show that there is little definitive information on the forms of degradation produced by these microorganisms in wood, although a consensus of opinion supports the view that many actinomycetes are cellulolytic. King and Eggins (13) in short-term, pure-culture studies, showed that members of the genus *Streptomyces* could rapidly colonize and extensively penetrate woody tissues.

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TABLE 1. — *Average weight loss produced by selected* Streptomyces *isolates after incubation on agar plates for 16 weeks.*[a]

Isolate	Douglas-fir	Red alder
	(%)	
Streptomyces A	1.7	0.7
Streptomyces B	1.5	0.6
Streptomyces C	1.3	+0.3[b]
Streptomyces D	1.1	0.0
S. xanthochromogenus	1.3	0.0
Control	0.0	+0.3[b]

[a]Each value is the average of 10 wood blocks, 5 in each of 2 agar plates.
[b]Weight gain.

TABLE 2. — *Average weight losses*[a] *in red alder colonized by monocultures of* Streptomyces *and various fungi in agar plates after various incubation periods.*

Test organism	4 weeks	8 weeks	12 weeks
		(%)	
Streptomyces A	0.9	1.3	1.8
S. xanthochromogenus	0.4	0.3	0.7
P. placenta	5.0	29.5	30.4
C. versicolor	27.6	50.0	54.8
C. globosum	0.1	+0.8[b]	+0.9[b]
Control	0.2	+0.3[b]	+0.4[b]

[a]Each value is the average of 10 wood blocks, 5 in each of 2 agar plates.
[b]Positive values represent weight gain resulting from experimental error.

Our study, therefore, focused on the extent to which these selected actinomycetes could cause wood degradation and the possible interactions between these organisms and fungi in the degradation of wood.

Materials and methods

Wood degradation

Small wood blocks measuring 10.0 by 10.0 by 5.0 mm were prepared from the sapwood of both Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco) and red alder (*Alnus rubra* Bong.), with the 10.0 by 10.0 mm face in the true transverse plane of the wood and the sides in true radial and tangential planes. The blocks were labeled, ovendried and weighed, and then autoclaved for 30 minutes at 121°C. Five *Streptomyces* isolates were used as test organisms. Four of the isolates were selected as the highest weight loss producers in a preliminary wood degradation test and maintained on starch-casein agar, and the fifth was an American Type Culture Collection (A.T.C.C.) culture (#19818) of *S. xanthochromogenus,* reported to produce significant weight losses in wood (1). These isolates were streaked onto the surfaces of petri plates that contained modified Kuster and Williams starch casein agar (14) from which the starch and casein were omitted. When the test organisms had grown, the plates were used to inoculate the wood blocks in such a fashion that five wood blocks were placed with transverse faces upon the actively growing streptomycete mycelium in each plate. Noninoculated control blocks of each type were also set up. All plates were incubated aerobically in darkness at 25°C in sterile polyethylene bags to minimize medium dehydration and contamination over the long test period of 24 weeks. Blocks were observed and removed at 4-week intervals for 24 weeks, ovendried to constant weight at 103°C, and weighed to determine their weight losses (Table 1).

Light microscopy, using interference contrast, was attempted on the sample material but was considered unfavorable for micromorphology of the colonization of wood by actinomycetes because of the small size of the hyphae and mechanical disruption during preparation.

Wafers were also prepared from wood blocks for scanning electron microscopy. Wood wafers were glued to electron microscopy stubs using silver oxide paste so that the split surfaces, formerly the interiors of colonized blocks, were uppermost and thus accessible for examination. They were then coated, under vacuum, with a film about 30 nm thick of gold-palladium alloy. The samples were examined in an ISI DS-130 scanning electron microscope.

Organism interaction

Agar plate decay tests were used to investigate the ability of *Streptomyces* to interact with decay and soft-rot fungi. The wood-destroying fungi were chosen as representatives of the three types of fungal attack: *Coriolus versicolor* (L.) Quel. (white-rot), *Postia placenta* Fr. (brown-rot), and *Chaetomium globosum* Kunze (soft-rot). Cultures of each were obtained from laboratory stocks and regenerated on malt extract agar. The streptomycetes used in conjunction with the above fungi were *Streptomyces* A (isolated from decayed red alder) and *Streptomyces xanthochromogenus* (obtained from A.T.C.C.). Cultures of each were maintained on starch casein agar (14).

One-cm cubes of untreated red alder sapwood blocks were used as test material. After all blocks had been cut, they were mixed and chosen at random. Accurately weighed samples (ovendry basis) were sterilized by autoclaving at a pressure of 20 psi for 30 minutes before being placed on agar in the agar plate decay chambers. Five treatments were used:

1. Blocks were placed in noninoculated chambers and then inoculated with monocultures of each streptomycete or fungus (Table 2).

2. Blocks were placed in noninoculated chambers and then inoculated simultaneously with monocultures of each combination of a streptomycete and a fungus (Table 3).

3. Blocks were inoculated with a streptomycete in a decay chamber and incubated at 25°C for 4 weeks, then aseptically transferred to another decay chamber containing a fungus.

4. Blocks were inoculated with a fungus in a decay chamber and incubated at 25°C for 4 weeks and then aseptically transferred to another decay chamber containing a streptomycete.

5. Blocks were placed in decay chambers and left noninoculated to provide control plates for each treatment.

Each treatment was replicated two times. Blocks were incubated in the dark at 25°C and then were measured for weight losses (ovendry basis) after incubation periods of 4, 8, and 12 weeks. Percentages of weight loss caused by a fungus alone were compared with percentages of weight loss caused by a fungus and a streptomycete together.

Results

Wood degradation and micromorphology

The weight loss produced in each block was calculated as the percentage of the original weight of the ovendry block after incubation with the microorganism. These weight losses were generally so low as to be considered insignificant by the usual criteria used in laboratory testing of Basidiomycetes (5%). Therefore, it appeared that the actinomycetes employed did not play a direct role in causing wood degradation. *Streptomyces xanthochromogenus* did not cause any significant weight losses in this study (Table 1), in contrast to an earlier report by Baecker (1) of significant attack in lime (*Tilia vulgaris* Hayne).

Scanning electron microscope examination of blocks demonstrated the occurrence of streptomycete mycelium in the woody tissue (Fig. 1) and provided evidence of pit penetration (Fig. 2). Penetration of both ray and longitudinal cells was extensive when wood blocks were exposed to actively growing streptomycete mycelium. There was no evidence of significant degradation or tissue dislocation in the blocks examined. No colonization, other than that of the test organisms, was noted. Only mild forms of tissue degradation, such as formation of what appeared to be shallow erosion troughs, were observed in both Douglas-fir and red alder. The soft-rot type cavities observed by Baecker (1) in lime wood blocks that had been colonized by *S. xanthochromogenus* were not observed in this study. Control blocks remained noncolonized throughout the experiment and no evidence of microbial colonization was evident when these were sectioned.

Organism interaction

Weight losses in red alder colonized by monocultures of *Streptomyces* and various fungi are shown in Table 2. Weight losses in red alder colonized by a combination of *Streptomyces* and various fungi are shown in Table 3. Weight losses produced by the Basidiomycetes (*P. placenta* and *C. versicolor*) alone were consistently higher than those produced by the combined attack of these Basidiomycetes with either of the streptomycetes in all treatments. This finding agreed with visual observations made of inoculated blocks, which found that the wood blocks were completely overgrown by the monocultures of fungi when no streptomycete was present. However, when blocks containing a streptomycete were placed on agar that was inoculated with a Basidiomycete, the Basidiomycete failed to reach the blocks, forming a zone of inhibition, which suggested the presence of antifungal compounds that diffused from the streptomycete-inoculated blocks. The ascomycete, *C. globosum*, did not cause any appreciable weight losses

TABLE 3. — *Average weight losses[a] in red alder colonized by combinations of* Streptomyces *and various fungi in agar plates after various incubation periods (weeks) following inoculation with the second organism.*

Test organisms	Simultaneous inoculation[b]			Inoculation with streptomycete before fungus[c]			Inoculation with fungus before streptomycete[d]		
	4	8	12	4	8	12	4	8	12
	(%)								
P. placenta and *Strep.* A	2.7	23.4	16.7	4.9	9.9	16.7	19.6	29.8	32.9
P. placenta and *Strep. xanth.*	1.2	10.7	19.3	2.3	11.3	13.9	13.4	26.6	31.2
C. versicolor and *Strep.* A	3.2	25.1	35.2	0.8	2.3	20.8	17.5	19.5	28.4
C. versicolor and *Strep. xanth.*	4.1	24.5	48.9	6.0	23.3	44.8	23.0	36.4	40.8
C. globosum and *Strep.* A	+2.1[e]	+1.0[e]	+0.2[e]	+0.1[e]	+0.4[e]	+0.1[e]	+0.3[e]	0.3	2.8
C. globosum and *Strep. xanth.*	+2.4[e]	+2.5[e]	+0.6[e]	+1.6[e]	+2.0[e]	+0.7[e]	+1.8[e]	2.1	0.6
Control	+1.7[e]	+0.5[e]	+0.7[e]	+1.9[e]	+0.6[e]	+1.4[e]	+1.7[e]	+0.6[e]	+0.8[e]

[a]Each value is the average of 10 wood blocks, 5 in each of 2 agar plates.
[b]Simultaneous inoculation of wood blocks with monocultures of *Streptomyces* and various fungi.
[c]Preinoculation and incubation of wood blocks with monocultures of *Streptomyces* for 4 weeks followed by reinoculation with monocultures of fungi.
[d]Preinoculation and incubation of wood blocks with monocultures of fungi for 4 weeks followed by reinoculation with monocultures of *Streptomyces spp.*
[e]Positive values represent weight gain resulting from experimental error.

Figure 1. — Colonization of Douglas-fir ray tissue by *Streptomyces* B (— = 3.75μ).

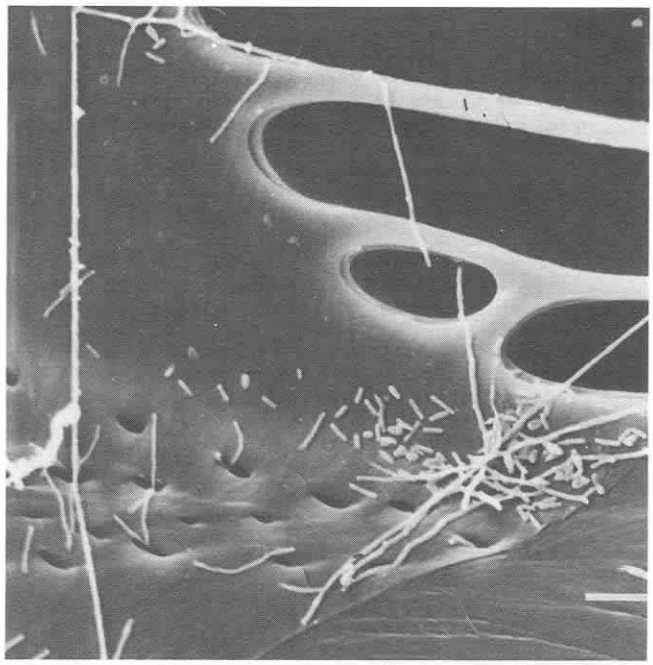

Figure 2. — Colonization of red alder vessel and penetration of pits by *Streptomyces* D (— = 3.82μ).

in monoculture or in combined attack with either of the streptomycetes in all treatments; its interaction with streptomycetes was considered inconclusive.

The rate of decay of the blocks was greatly reduced when the test blocks were inoculated with the monocultures of streptomycetes prior to inoculation with the monocultures of Basidiomycetes. The rate of decay was reduced less with simultaneous inoculation, and still less with Basidiomycetes before streptomycetes. In all treatments, the streptomycetes tested were effective in reducing the rate of decay caused by the Basidiomycetes, however, *S. xanthochromogenus* was more effective in reducing the rate of decay caused by *P. placenta,* and *Streptomyces* A was more effective in reducing the rate of decay caused by *C. versicolor.*

Discussion

On the basis of the slight weight losses produced in both softwood and hardwood, there is no experimental evidence that the actinomycetes isolated in the present study play a direct role in causing wood degradation. All the colonization patterns observed were considered to be consistent with the hypothesis that cell contents were utilized by the microorganisms, with no associated degradation of the wood cell walls. The soft-rot-type cavities observed by Baecker (1) in lime (*Tilia vulgaris* Hayne) wood blocks that had been colonized by *S. xanthochromogenus* were not observed in this study. This lack of evidence of cell wall degradation by streptomycetes held true even after long incubation periods. Similarly, studies by Eaton and Dickinson (7) have shown that after incubation periods in excess of 18 months in a marine environment, degradation produced by an unidentified organism (suggested to be a streptomycete) was slight. The insignificant levels of degradation produced by the test organisms in this study are consistent with individual observations by other researchers using monocultures of actinomycetes in wood decay tests (1,5,6,9).

Since these actinomycetes themselves did not appear to degrade wood significantly, it was considered possible that their consistent isolation from decayed wood and their apparent success in colonizing wood may have been due to their ability to coexist with the rest of the wood microflora. The results of the interaction portion of this study suggest that the isolated streptomycetes may reduce the rate of decay caused by the wood decay fungi.

This study showed that the slow-growing isolates of *Streptomyces* seem to reduce the rate of wood decay by producing antifungal compounds and possibly by directly competing with fungi for nutrients. The *Streptomyces* may also have an effect on the linear growth of the microorganisms. Working on a similar project, Jacquiot (11) observed that *C. versicolor* was particularly resistant to bacterial inhibition and that, of the bacteria he tested, only one organism was effective against both this fungus and *C. globosum*. Both of the streptomycetes used in our study were effective against both types of fungi. In initial tests, Jacquiot (11) and Rypacek (16) were not able to obtain effective inhibition within the wood when material was impregnated with a bacterial culture and immediately placed on fungal mycelium. Their impressions were that the decay fungus, under these conditions, was able to successfully compete with the bacterium. The tests described here indicate that i. hibition results, not only from direct competition between microorganisms within the wood, but to a greater extent from the production of antifungal compounds.

Conclusions

1. Monocultures of all streptomycetes tested colonized both Douglas-fir and red alder, but weight losses produced in the two wood types by the microorganisms were insignificant.

2. Actinomycetes appear to be among the colonizers of wood in soil contact, but, on the basis of the slight weight losses produced in both hardwood and softwood, it appears that the actinomycetes isolated in this study do not play a direct role in causing wood degradation.

3. Streptomycetes have the ability to reduce the rate of biodeterioration of wood by fungi.

4. The use of streptomycetes as a means of retarding wood decay may be considered because of their ability to produce antifungal compounds, which may inhibit fungi, and because they compete directly with the fungi for available nutrients.

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